Peanut Web Blotch: II Symptoms and Host Range of Pathogen[1]

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ABSTRACT

Web blotch, caused by *Phoma arachidicola*, has varied in severity from year to year in Texas, depending on survival of inoculum on peanut crop residue, presence of susceptible peanut cultivars and suitable environmental conditions, especially favorable temperature and high relative humidity. The web and blotch symptoms may develop in sequence on the same leaflet or independently on different leaflets. At relative humidities below 80% within the plant canopy, the fungal hyphae grew under the leaf cuticle causing a web-type symptom. At relative humidities above 90%, hyphal strands grew more repidly and branched extensively in the leaf resulting in the blotch-type symptom. *P. arachidicola* isolates from South Africa, Argentina, and the USA were all pathogenic and caused similar symptoms. Of 32 legumes, peanut, sweetclover, hairy vetch and alfalfa were the most susceptible. Symptoms on hosts other than peanuts consisted of small necrotic spots. A hypersensitive reaction developed on eight legumes. Typical web blotch symptoms were observed only on peanuts in the field.

Key Words: *Arachis hypogaea*, foliar diseases, groundnut, *Phoma arachidicola*

A peanut disease with both web and blotch symptoms on leaflets was first observed in the United States by Arthur L. Harrison and Dugan Wells near Uvalde, Texas in June 1972. In subsequent isolation and inoculation studies, Taber (11) determined the disease to be caused by an *Ascochyta* sp. A similar disease has been reported in several other countries where peanuts are grown (2, 4, 7, 10, 12). The first report of a similar disease was by Woronichin (21) in Russia in 1924, and he classified the causal agent as *Ascochyta arachidis* Woron. In another Russian report, Khorhryakov (8) described a peanut foliar disease with blotch-type symptoms. In 1962, Cruz *et al.* (5) described a peanut foliar disease in Brazil they termed "muddy spot". They also suggested the disease was caused by an *Ascochyta* sp. In 1969 Frezzi (7) reported on a peanut foliar disease with blotch-type symptoms in Argentina and three years later Rothwell (14) reported on a similar peanut disease in Zimbabwe, Rhodesia. In 1975, Marasas *et al.* (10) reported a peanut disease in South Africa similar to that reported in Texas and Argentina. They re-described the web blotch fungus as *Phoma arachidicola* Marasas, Pauer, and Boerema, in keeping with the newer concept of the generic differences between *Ascochyta* and *Phoma* (10). Later, Alcorn *et al.* (2) reported on a similar peanut disease in Australia that they called "net blotch". Recently, Smith *et al.* (16) reported that virginia and runner market-type peanut cultivars are more resistant to web blotch than the spanish market-type cultivars. The purpose of this study was to determine environmental conditions that favor web blotch development, the source of initial inoculum, pathogenicity of *Phoma* isolates form different geographical areas, and susceptibility of other legumes. Preliminary reports have been presented (11, 12).

Materials and Methods

Web blotch occurrence and severity have been observed in the field each year (1972-1982) in the major peanut production areas of Texas. Each time disease symptoms were observed the environmental conditions and peanut cultivar were recorded.

To determine the source of primary inoculum, the survival of *P. arachidicola* on crop residue was monitored in South Texas during the winters of 1972 and 1973. Diseased crop residue was caged on the soil surface and leaves were collected periodically. Conidia and ascospores were washed from leaves with sterile distilled water, the washings atomized onto healthy leaves, and the plants shaded and covered with plastic bags for 24-48 h in a greenhouse and observed periodically for the development of disease symptoms. Additional observations were made in peanut fields early in the spring to determine when disease development was initiated. Leaflets from volunteer plants with web blotch symptoms were collected in the field, cleared, stained with cotton blue, and examined for spores and mycelial growth.

Several *P. arachidicola* isolates were tested for pathogenicity and capability of causing typical web-blotch symptoms. Isolations were made from leaflets collected in Florida by D. H. Smith (isolate G), in Oklahoma by D. H. Smith, (isolate 0), in Argentina by M. J. Frezzi (isolate Ar), in South Africa by G. H. Boerema (isolate S), and in Texas by G. L. Philley (isolate P). Each isolate was grown on potato dextrose agar, malt-extract agar, and autoclaved peanut leaves. Inocula consisted of conidia, chlamydospores, and ascospores washed from agar plates with sterile distilled water containing a few drop of Triton CS-7. The inoculum was applied to healthy leaflets of 60 day old Starr and Florunner cultivars. Each isolate was applied to the leaves with a cotton swab and the inoculated plants placed in a 20-25 C moist chamber (95-100% R. H.) for 48 h. Plants were then transferred to greenhouse benches and symptom expression was monitored for several weeks.

Since *Phoma* species commonly parasitize legumes (1, 6, 17, 18), a host range study for *P. arachidicola* was conducted on 32 legumes: alyceclover (*Alysicarpus vaginalis* (L.) D. C.), peanut (*Arachis hypogaea* L.), milkvetch (*Astragalus* sp.), crown vetch (*Coronilla varia* L.), greenleaf desmodium (*Desmodium intortum* Urb.), silverleaf desmodium (*D. uncinatum* Burk.), soybean (*Glycine max* (L.) Merr.), sweet pea (*Lathyrus odoratus* L.), striate lespedeza (*Lespedeza striata* (Thunb.) H. and A.), Korean lespedeza (*L. stipulacea* Maxim.), Miles lotononis (*Lotononis bainesii* Bar.), birdsfoot trefoil (*Lotus corniculatus* L.), Marsfield big trefoil (*L. pedunculatus* Cav.), narrow leaf trefoil (*L. tenuis* Waldst. and Kib.), alfalfa (*Medicago sativa* L.), Israel sweet clover (*Melilotus alba* Desr. var *annua* Coe), siratro (*Phaseolus atropurpureus* D. C.),

[1]Contribution from the Texas Agricultural Experiment Station, Texas A&M University, College Station, TX. TA No. 20487 Part of a Ph. D. dissertation submitted by George L. Philley.

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pinto bean (*P. vulgaris* L.), English pea (*Pisum sativum* L.) Austrian winter pea (*P. sativum* L. var *arvense* (L.) Poir), stylo (*Stylosanthes humilis* Hbk.), Berseem clover (*Trifolium alexandrinum* L.), alsike clover (*T. hybridum* L.), crimson clover (*T. incarnatum* L.), ball clover (*T. nigrescens* Vir.), red clover (*T. pratense* L.), white Dutch clover (*T. repens* L.), Persian clover (*T. resupinatum* L.), subterranean clover (*T. subterraneum* L.), Yuchi arrowleaf clover (*T. vesiculosum* Savi.), hairy vetch (*Vicia villosa* Roth), and cowpea (*Vigna unguiculata* (L.) Walp.).

Ten replicate test plants of each species were grown in 15-cm plastic pots. Inocula were prepared from all *P. arachidicola* isolates grown on autoclaved peanut leaflets on water agar as follows: autoclaved leaflets were inoculated with each *Phoma* isolate, incubated 3 days at 20 C in the dark, and then exposed 24 h to near-ultraviolet light to trigger uniform pycnidial formation. After 10 days, leaflets were taker from the water agar and spores removed in sterile distilled water containing several drops of Triton CS-7.

Spore suspensions for plant-host range inoculations consisted of a mixture of equal portions of the five *Phoma* isolates. The spore suspension was applied to test plants with a cotton swab repeatedly dipped in the suspension. Following inoculation, all plants were placed in a moist chamber at 21 ± 3 C for 3 days and then either removed to a growth chamber or greenhouse. The growth chamber was programed for a 12-h light cycle at 22 C and a 12-h dark cycle at 20 C. Relative humidity fluctuated from 70-90% and 80-95% during the light and dark cycles, respectively. Plants were examined 14 and 24 days after inoculation. Disease severity was rated using a scale of 1 to 5 where 1 indicated no visible disease symptoms and 5 indicated severe leaf disease symptoms. Leaflets from the test plants were also cleared, stained with cotton blue, and examined to detect spore germination and germ tube penetration. The degree of penetration and subcuticular fungal growth was rated on a 1 to 5 scale where 1 indicated no growth and 5 indicated extensive growth. Hypersensitive-like reactions were also recorded. To verify that *P. arachidicola* caused the disease, leaflets from plants exhibiting symptoms were cut in small sections, surface-sterilized with 0.5% NaOCl for 1.5 min, and plated on malt-extract agar for re-isolation of the pathogen.

Results and Discussion

Occurrence and Environmental Conditions

The incidence of peanut web blotch in Texas during the period 1972-1982 has been sporadic. It was most severe on spanish varieties in South Texas during June and July of 1972, 1974, and 1976. Web blotch has been of minor importance in North Central Texas, generally occurring late in the growing season. Plants of all ages are susceptible. On the West Texas High Plains, peanut web blotch has gradually become more severe in recent years. It develops there from midseason to harvest. The severity of the disease in any one year is closely related to the presence of susceptible peanut cultivars, inoculum availability and favorable environomental conditions. Periods of cloudy weather with frequent rains and temperatures (15-30 C) favor fungal activity (3). The most severe disease outbreaks occurred during and immediately after periods of extended thundershowers when temperatures remained below 30 C and cloud cover reduced light intensity.

In South Texas, where web blotch was severe in 1972 and 1973, the fungus was able to overwinter within crop residue. Both conidia and ascospores were retrieved from overwintered crop residue. Inoculation of plants with these spores resulted in the development of typical disease symptoms. Field observations during May 1973 revealed the presence of typical web blotch symptoms on 30-day-old volunteer plants in South Texas peanut fields. The following year (March 1974), volunteer plants exhibited web blotch symptoms during an unusually warm (15-26 C), damp period. Microscopic examination of leaflets from volunteer plants in early spring revealed that ascospores were the primary infective propagules.

All varieties of peanuts grown in Texas are susceptible to *P. arachidicola*; however, differences in susceptibility were evident. Florunner was less susceptible when compared to Starr. In other studies, Smith *et al.* (16) reported that Florunner, GK-53, Golden I, Ga 123, and GK-19 have some degree of resistance. Phipps (13) evaluated 16 peanut cultivars in Virginia and reported that Florigiant, Argentine, and Chico were more susceptible to *P. arachidicola* than Florunner and that NC 3033 was the most web blotch resistant cultivar tested.

Typical symptoms of web blotch are illustrated in Fig. 1. Both the web and blotch symptoms typically appear first on the adaxial leaf surface and can develop independently or simultaneously. The web symptom develops when environmental conditions are less conducive for extensive disease development. Each strand of the web is associated with a single hyphal strand growing immediately below the leaf cuticle. The web symptom is caused by necrosis of epidermal cells adjacent to the advancing hyphae. Primary controlling factors in disease expression are leaf wetness and relative humidity of the microenvironment. When moist conditions prevail, hyphal growth is more extensive with considerable branching and penetration of the palisade cells. Extensive damage of the leaf tissue results in the development of the blotch-type symptom. Sometimes the web-and blotch-type symptoms develop in sequence while at other times one symptom predominates.

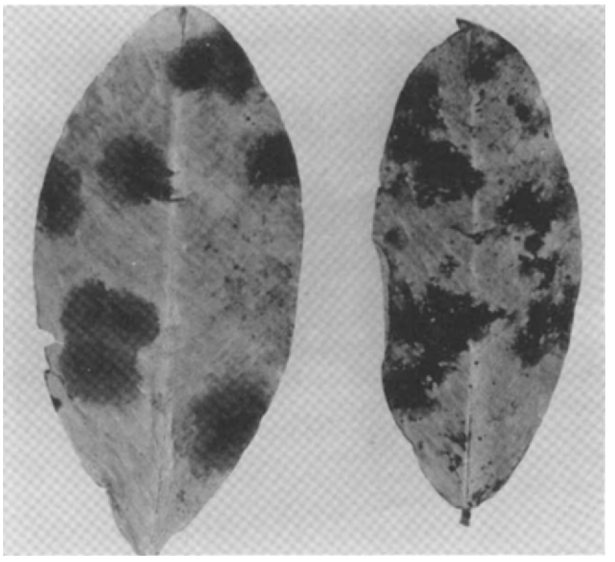

Fig. 1. Peanut leaflets with blotch (left) and web (right) disease symptoms.

In the field, web blotch was more severe during high relative humidity periods and more extensive defoliation occurred following showers or irrigations. Up to 40-60% of the leaves have been observed to abscise. When dry,

warm weather followed severe web blotch development, disease progression slowed, a new set of leaves formed and flowering resumed. New leaves aided the plant in filling pods formed earlier.

Pycnidia of *P. arachidicola* were seldom observed on diseased leaves attached to the plant. When pycnidia did form on attached leaves they occurred in the most necrotic areas of the blotches. After infected leaves abscised and fell to a damp soil surface, abundant pycnidia and/or pseudothecia formed in the leaf tissue during fall and winter months. Pseudothecia also formed on raised portions of leaves in contact with a moist soil surface. Chlamydospores developed on decaying leaves in the field and were observed to germinate and cause infection. Each of these propagules was shown to be effective in reproducing the disease (2). Phipps (13) in Virginia has also reported that chlamydospores were effective inocula.

Pathogenicity of isolates on peanuts

All *Phoma* isolates from South Africa, Argentina and United States caused similar disease symptoms on 30-60 day old leaflets of both Starr and Florunner cultivars. Spore germination and cuticle penetration occurred on leaflets of each peanut variety; however, hyphal growth within Florunner leaflets was slower. Initial symptoms were noted at the microscopic level within 4 days after inoculation and macroscopic symptoms 1-8 days later, depending on the ambient air temperature and relative humidity. Plants in the growth chamber at 70% RH exhibited more rapid defoliation compared to plants grown in the greenhouse at 60% RH. After 20 days in the growth chamber, 40.8% of the leaflets on Starr plants had abscised. Disease development was slower on Florunner in growth chambers, greenhouse, and field. These observations on symptom expression agree with reports by other researchers in Brazil (5), Argentina (7), Rhodesia (Zimbabwe) (14), South Afirca (10) and Australia (2).

Host range

Results from the host range study for *P. arachidicola* revealed that eleven of 32 legume test entries developed leaf disease symptoms. Legumes that had expanding lesions (Table 1) were peanut, alfalfa, Israel sweet clover, berseem clover, alsike clover, Yuchi arrow leaf clover, and hairy vetch. Symptoms on plants other than peanuts consisted of small necrotic spots similar to those reported by Latch and Hansen (9) as caused by *Mycosphaerella lethalis* Stone *(Ascochyta meliloti).* On sweet clover and hairy vetch, a large number of small spots developed. On alfalfa and the other clovers, a number of spots developed and these spots remained relatively small (1-4 mm). Leaf spots developed more rapidly on older, mature leaves. Microscopic observations of cleared leaves indicated that fungal growth within leaves was most extensive in peanut, alfalfa, Israel sweet clover, and hairy vetch. Epidermal cell penetration was evident in cleared leaflets of these potential hosts but less extensive than in peanut leaflets. Hyphal ramification within the palisade cell layer was most pronounced in peanut, sweet clover, and hairy vetch.

Mycosphaerella lethalis has been reported as a pathogen on soybeans and red clover. In these studies, *P. arachidicola* did not parasitize soybeans or red clover; however, Smith and McGee (15) have reported that *P. arachidicola* caused necrotic lesions on Semmes, McNair 800, and Bragg soybean varieties.

Table 1. Relative susceptibility of legumes to *Phoma arachidicola*

	Disease Severity[a]	Penetration and growth[b]	Hypersensitive reaction
Alysicarpus vaginalis	2	3	-
Arachis hypogaea	5	5	-
Astragalus sp.	1	1	-
Coronilla varia	1	1	-
Desmodium intortum	1	2	-
D. uncinatum	1	1	-
Glycine max	1	1	-
Lathyrus odoratus	1	1	-
Lespedeza striata	1	1	-
L. stipulacea	-[c]	2	+
Lotononis bainesii	1	1	-
Lotus corniculatus	1	2	-
L. pedunculatus	-	2	+
L. tenuis	-	2	+
Medicago sativa	-	4	+
Melilotus alba var annua	3	5	-
Phaseolus atropurpureus	1	1	-
P. vulgaris	1	1	-
Pisum sativum	-	1	+
P. sativum var arvense	-	1	+
Stylosanthes humilis	1	1	-
Trifolium alexandrinum	2	3	-
T. hybridum	2	3	-
T. incarnatum	1	1	-
T. nigrescens	2	2	-
T. pratense	1	1	-
T. repens	-	2	+
T. resupinatum	-	2	+
T. subterraneum	1	3	-
T. vesiculosum	2	3	-
Vicia villosa	3	4	-
Vigna unguiculata	1	1	-

[a] Disease severity was rated on a 1 to 5 scale. 1 indicated no disease symptoms and 5 severe symptoms.

[b] Penetration and growth was rated on a 1 to 5 scale. 1 indicated no penetration and 5 indicated extensive growth within the leaf tissue.

[c] Plants which exhibited a hypersensitive reaction were not rated for disease severity.

A hypersensitive-like reaction developed on eight of the legumes tested. Following penetration of the cuticle, fungal hyphae grew just under the cuticle for a few mm beyond the point of penetration and ceased growth. In some cases, intercellular penetration was evident followed by intracellular invasion of the palisade layer of leaf tissue. On *Pisum,* numerous small dark specks developed that failed to enlarge, a typical hypersensitive-type reaction. Germination of fungal spores was observed around necrotic spots on *Pisum* but no epidermal penetration was observed. It appeared that penetration had started but ceased when epidermal and palisade cells died. On alfalfa, a hypersensitive-like reaction was observed.

A comparison between growth chamber and greenhouse-grown legumes indicated that there was no noticeable difference in symptom development in these two environments, with the exception of symptoms on peanuts. Web-type symptoms developed on peanuts in 14 days in a growth chamber and 15 days in a greenhouse at lower relative humidities. On other hosts, lesion development required 14 days regardless of where the plants were grown.

Phoma arachidicola was isolated from all hosts on which obvious symptoms developed. Pycnidia were observed

on abscised sweet clover and hairy vetch leaves from moist soil surfaces.

The growth of *P. arachidicola* under the cuticle of peanut leaflets appears to be a distinct feature of the growth habit of this fungus. An examination of inoculated leaves of sweet clover and hairy vetch revealed epidermal penetration, subcuticular growth of the hyphal strands, and later hyphal penetration of the palisade cells.

On other potential host plants, *P. arachidicola* was a relatively weak pathogen. On alfalfa, Berseem clover, White Dutch clover, alsike clover, ball clover, and Yuchi arrow leaf clover, only the older leaves were susceptible. Hyphal penetration was not observed in the young leaves.

Examination of cleared leaves from greenleaf desmodium, Korean lespedeza, subterranean clover, white Dutch clover, alfalfa, birdsfoot trefoil, narrow leaf trefoil, Marsfield big trefoil and Persian clover revealed that epidermal penetration and/or subcuticular growth of hyphae had occurred. There was no evidence of hyphal penetration or a hypersensitive reaction on the other 13 potential hosts.

These studies have revealed that peanuts are the primary host of *P. arachidicola;* however, other hosts may serve as sources of primary inoculum. Web blotch continues to be an important disease in peanut producing areas where spanish market-type cultivars are produced.

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Accepted March 28, 1986